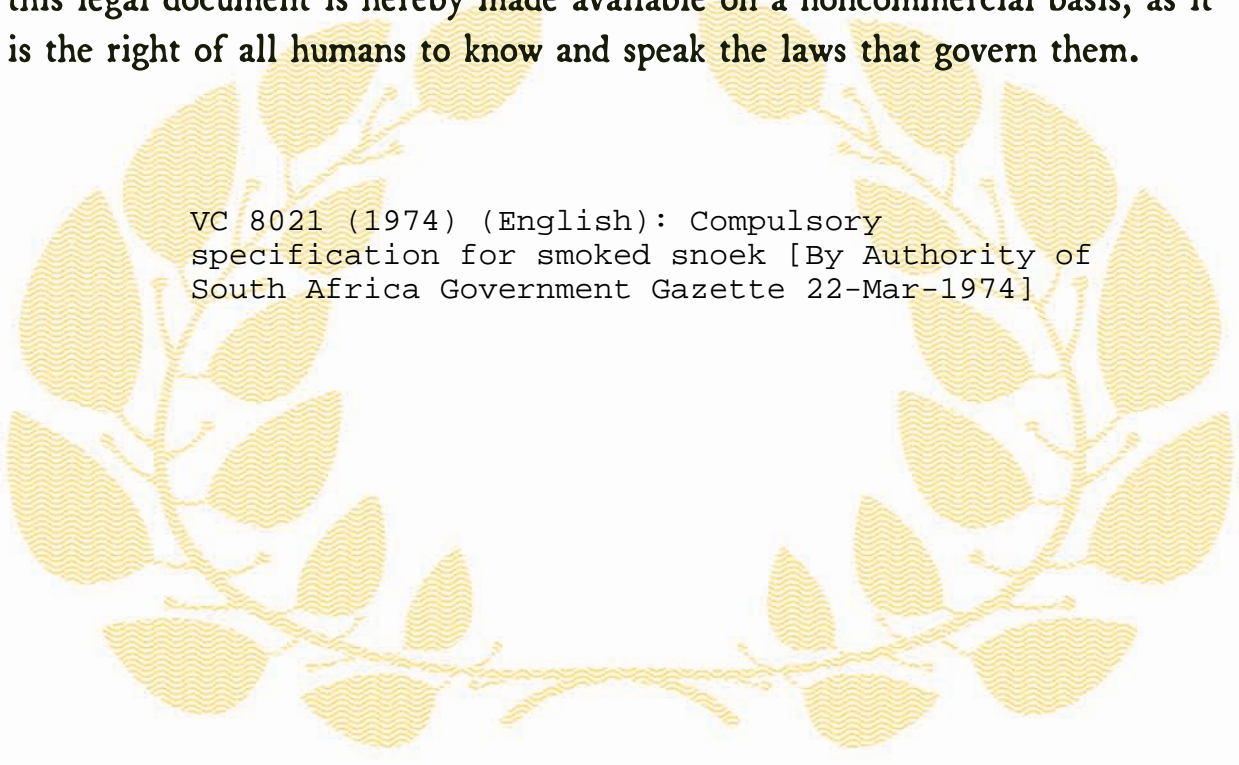




# *Republic of South Africa*

## EDICT OF GOVERNMENT

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VC 8021 (1974) (English): Compulsory  
specification for smoked snoek [By Authority of  
South Africa Government Gazette 22-Mar-1974]



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**Compulsory Specification for/  
Verpligte Spesifikasie vir**

**Smoked snoek/  
Gerookte snoek**

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22 Maart 1974

UDC/UDK 664

**VC 8021**



Vc 8021

STANDARDS ACT, 1962  
(Act No. 33 of 1962)

COMPULSORY STANDARD SPECIFICATION

for

SMOKED SNOEK

as published by Government Notice No. 474 of  
22 March 1974

WET OP STANDAARDE, 1962  
(Wet No. 33 van 1962)

VERPLIGTE STANDAARDSPESIFIKASIE

vir

GEROOKTE SNOEK

soos afgekondig by Goewermentskennisgewing No. 474 van  
22 Maart 1974

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## DEPARTEMENT VAN NYWERHEIDSWESE

No. 474.

22 Maart 1974

### WET OP STANDAARDE, 1962

#### VERPLIGTE STANDAARDSPESIFIKASIE VIR GEROOKTE SNOEK

Op aanbeveling van die Raad van die Suid-Afrikaanse Buro vir Standaarde en kragtens die bevoegdheid my verleen by artikel 15 van die Wet op Standaarde, 1962 (Wet 33 van 1962), verklaar ek, Jan Christiaan Heunis, Adjunk-minister van Ekonomiese Sake, hierby die standaardspesifikasie in die Bylae vervat tot verpligte standaardspesifikasie vir gerookte snoek, met ingang van die datum twee maande na publikasie van hierdie kennisgewing.

J. C. HEUNIS, Adjunk-minister van Ekonomiese Sake.

#### BYLAE

#### VERPLIGTE STANDAARDSPESIFIKASIE VIR GEROOKTE SNOEK (Metrieke Eenhede)

##### 1. BESTEK.

1.1 Hierdie spesifikasie dek die vervaardiging, produksie, verwerking of behandeling van die gekookte produk, gerookte snoek (*Thyrstites atun*).

##### 2. WOORDBEPALING.

2.1 Die volgende woordbepalings geld vir die doel van hierdie spesifikasie:

*Aanneemlik.*—Aanneemlik vir die owerheid wat hierdie spesifikasie toepas.

*Fabriek.*—Enige perseel waar die produk voorberei of verwerk word of albei. Vir sover die vereistes van hierdie spesifikasie toepaslik is, dek hierdie woordbepaling ook fabriekskepe en vissersbote wat die produk vir rokingsdoeleindes hanteer.

*Gerookte snoek.*—Die voedsel vir menslike verbruik wat verkry word deur die voorbereiding en warmroking van snoek.

*Halofiele.*—Bakterieë en skimmel wat in die aanwesigheid van hoë konsentrasie gewone sout ontwikkel en vermenigvuldig met die gepaardgaande bederf van die gesoute produk. Hierdie omskrywing sluit mikroörganismes in wat verkleuring en die ontstaan van rooi, pienk en vaal-bruin pigmente veroorsaak.

*Inspekteur.*—'n Inspekteur wat kragtens artikel 18 van die Wet op Standaarde aangestel is.

*Produk.*—Snoek wat gerook gaan word of wat vir rook voorberei word of wat gerook word of wat gerook is, soos blyk uit die sinsverband.

##### 3. ALGEMENE VEREISTES VIR DIE FABRIEK EN WERKNEMERS.

3.1 ALGEMENE VEREISTES VIR DIE FABRIEK.  
—Daar moet aan die vereistes van die Wet op Fabrieke,

## DEPARTMENT OF INDUSTRIES

No. 474

22 March 1974

### STANDARDS ACT, 1962

#### COMPULSORY STANDARD SPECIFICATION FOR SMOKED SNOEK

On the recommendation of the Council of the South African Bureau of Standards and under the powers vested in me by section 15 of the Standards Act, 1962 (Act 33 of 1962), I, Jan Christiaan Heunis, Deputy Minister of Economic Affairs, hereby declare the standard specification contained in the Schedule to be a compulsory standard specification for smoked snoek, with effect from the date two months after the publication of this notice.

J. C. HEUNIS, Deputy Minister of Economic Affairs.

#### SCHEDULE

#### COMPULSORY STANDARD SPECIFICATION FOR SMOKED SNOEK (Metric Units)

##### 1. SCOPE.

1.1 This specification covers the manufacture, production, processing, or treatment of the cooked product, smoked snoek (*Thyrstites atun*).

##### 2. DEFINITIONS.

2.1 For the purposes of this specification the following definitions shall apply:

*Acceptable.*—Acceptable to the authority administering this specification.

*Factory.*—Any premises in which the product is prepared or processed or both. To the extent to which the requirements of this specification can be applied, this definition includes factory ships and fishing vessels handling snoek for the purposes of smoking.

*Halophiles.*—Bacteria and moulds capable of development and proliferation in the presence of high concentrations of common salt wit accompanying spoilage of the salted product. The definition includes the discoloring micro-organisms commonly known as "red bacteria", "pinking bacteria" and "dun".

*Inspector.*—An inspector appointed in terms of section 18 of the Standards Act.

*Product.*—Snoek intended for smoking or in the course of preparation for smoking or in the course of being smoked or after having been smoked, as indicated by the context.

*Smoked snoek.*—The article of food for human consumption obtained by the preparation and hot smoking of snoek.

##### 3. GENERAL REQUIREMENTS FOR THE FACTORY AND FOR EMPLOYEES.

3.1 GENERAL REQUIREMENTS FOR THE FACTORY.—The requirements of the Factories, Machinery

Masjinerie en Bouwerk voldoen word. Daarbenewens moet die fabriek, gereedskap, bevroings- en koelkamergebiede en water wat by die voorbereiding van die produk gebruik word, aan die volgende vereistes voldoen:

**3.1.1 Konstruksie van en toestande in die fabriek.**

3.1.1.1 Die dak moet weerbestand wees en indien daar 'n plafon is, moet dit glad en stofdig wees.

3.1.1.2 Die vloer moet van waterdigte materiaal gemaak wees. Die oppervlakafwerking daarvan moet behoorlik skoongemaak kan word. Dit moet 'n behoorlike val hê en moet in buite-afvoerslootjies dreineer wat met rioolpype of afvoerkanale verbind is. Die vloer moet gedurende produksietye skoongehou word deur dit gereeld te vee en met water af te spoel, en daar moet gesorg word dat vuilnis nie in afvoerkanale ophoop nie. Die vloere en afvoerkanale moet na afloop van produksiewerksaamhede deeglik skoongemaak word. Indien nodig, moet plankmatte vir werkers voorsien word.

3.1.1.3 Die binnevlakke van die mure van verwerkingskamers moet waterdig wees en moet tot 'n hoogte van minstens 1,8 m bokant die vloervlak 'n gladde, ligkleurige, wasbare afwerking hê. Die mure moet, indien nodig, onmiddellik na elke dag se werksaamhede deeglik gewas word.

3.1.1.4 Rommel, afval en oorloop moet dadelik en doeltreffend op 'n higiëniese wyse, indien moontlik met meganiese middele, weggeruim word.

3.1.1.5 Die algemene verligting moet van so 'n aard wees dat dit doeltreffende voorbereiding, verwerking en skoonmaak toelaat.

3.1.1.6 Die ventilasie moet oortollige stoom verwyder en moet die vorming van kondensaasie verhoed wat in die grondstowwe of die produk of op uitrusting wat daarmee in aanraking kom, kan val. Indien nodig, moet die natuurlike ventilasie op meganiese wyse aangevul word.

3.1.1.7 Doeltreffende maatreëls moet getref word om skimmelgroei te verhinder en om stof, afskilferende verf en ander los materiaal wat van mure, plafonne en bostrukture in verwerkings- en bewaarkamers op die produk kan val, te verwyder.

3.1.1.8 Doeltreffende maatreëls moet getref word om vlieë en ander insekte uit die fabriek te hou.

3.1.1.9 Die fabriek waarin die produk voorberei of verwerk word, moet knaagdierdig wees en moet vry van knaagdiere gehou word.

3.1.1.10 Insek- en knaagdierdoders en chemiese skoonmaakmiddels mag nie gebruik word terwyl voorbereidings- of vervaardigingswerksaamhede aan die gang is nie. Werkvlakke moet vry van insek- en knaagdierdoderes residu's gehou word. Insek- en knaagdierdoders en skoonmaakmiddels mag nooit met toedraaipapier, houers, grondstowwe of die produk in aanraking kom nie.

3.1.1.11 Enige afvoerstelsel by die kaai en enige vervoerstelsel na die fabriek, met inbegrip van hysbakke, moet voor en na gebruik skoongemaak word en staande water en ou materiaal moet daaruit verwyder word. Bewaartenks moet op dieselfde wyse behandel word.

3.1.1.12 Geen fabriekskoorsteen, berokingskamer of motoruitlaatpyp mag rook of dampe afgee in hoeveelhede of op 'n wyse wat aanstootlik, of skadelik of gevaarlik vir die gesondheid is of wat in enige stadium van die voorbereiding van die produk besoedeling veroorsaak nie.

3.1.1.13 Verwerkingsinstallasies vir die vervaardiging van nuwe produkte wat 'n nadelige uitwerking op die produk kan hê, moet doeltreffend van die fabriek geskei wees. Afval moet op higiëniese wyse van die verwerkingsgebied verwyder word, en houers met afval wat uit die fabrieksterrein geneem moet word, moet goed van die verwerkingsgebied geskei wees.

and Building Work Act shall be complied with. In addition the factory, equipment, freezing and cold storage facilities, and water used in the preparation of the product shall comply with the following requirements:

**3.1.1 Construction of and conditions in the factory.**

3.1.1.1 The roof shall be weatherproof and the ceiling, if present, smooth and dustproof.

3.1.1.2 The floor shall be constructed of material impervious to water. It shall have a surface finish which will permit proper cleaning. It shall be adequately graded and drained to external gullies connected to sewers or drains.

During production periods the floor shall be kept clean by regular sweeping and flushing with water and refuse shall not be permitted to accumulate in drainage channels. At the close of operations, thorough cleaning of floors and drainage channels shall take place. Where necessary, duckboards shall be provided for workers.

3.1.1.3 The inside surfaces of the walls of processing rooms shall be impervious to water and shall have a smooth light-coloured washable finish to a height of at least 1,8 m above floor level. They shall, where necessary, be thoroughly washed immediately after each day's operation.

3.1.1.4 Litter, waste and overflow shall be disposed of promptly and efficiently in a sanitary manner, where possible by mechanical means.

3.1.1.5 General illumination shall permit effective preparation, processing and cleaning.

3.1.1.6 The ventilation shall effect the removal of excess steam and shall prevent the formation of condensate which may fall into raw materials or the product or on equipment coming into contact with them. Natural ventilation shall be augmented, if necessary, by mechanical means.

3.1.1.7 Effective measures shall be taken to inhibit mould growth and to remove dust, flaking paint, and other loose or detachable material liable to fall on the product from walls, ceilings and overhead structures in processing and storage rooms.

3.1.1.8 Effective measures shall be taken to keep the factory free from flies and other insects.

3.1.1.9 The factory in which the product is prepared or processed shall be rodentproofed and shall be kept free from rodents.

3.1.1.10 Insecticides, rodenticides and cleaning chemicals shall not be used while preparation or manufacturing operations are in progress. Working surfaces shall be kept free from insecticidal and rodenticidal residues. Insecticides, rodenticides and cleaning chemicals shall at no time come into contact with wrappers, containers, raw materials or the product.

3.1.1.11 Any discharge system at the jetty and any conveyance system to the factory, including elevators, shall be cleared of stagnant water and stale material and shall be cleaned before and after use. Holding tanks shall be similarly treated.

3.1.1.12 No factory chimney, smoke-room or motor exhaust shall emit smoke or fumes in a quantity or in a manner that is offensive or injurious or dangerous to health or causes contamination at any stage in the preparation of the product.

3.1.1.13 Processing plants for the manufacture of by-products which may deleteriously affect the product shall be effectively separated from the factory. Offal shall be removed from the processing area in a hygienic manner and containers for offal awaiting removal from the factory area shall be well separated from processing areas.

3.1.1.14 Voldoende wasbakke of -trôe met aanneemlike seep of detergent, 'n aanneemlike ontsmettingsmiddel en voldoende skoon warm en koue lopende water of skoon warm water vir handewas, moet op gerieflike plekke in al die voorbereidings- of verwerkingsafdelings van die fabriek waar daar met die hand aan die onbeskermd produk geraak word, verskaf word. Indien die hande na die was afgedroog moet word, moet weggooihanddoeke of ander aanneemlike middele om die hande af te droog, verskaf word.

3.1.1.15 Die fabriek moet te alle tye in 'n higiëniese toestand gehou word. Diere mag in geen deel van die fabriek as sulks toegelaat word nie.

3.1.1.16 Met betrekking tot die hantering, vervoer, voorbereiding, rook, verpakking, vries (indien toepaslik) en bewaring van die produk, mag geen handeling plaasvind of toestand heers wat nadelig vir die produk is nie.

### 3.1.2 Uitrusting.

3.1.2.1 Alle installasies, uitrusting en gereedskap wat met die produk in aanraking kom, moet van materiaal met 'n gladde oppervlak en wat bestand is teen korrosie deur pekkel gemaak wees, moet 'n higiëniese ontwerp hê en moet sodanig gemaak wees dat dit maklik is om die uitrusting, sowel as die plekke waarop dit staan, skoon te maak en te steriliseer. Lood en loodlegerings, uitgesonderd soldeersel, mag nie vir die konstruksie van uitrusting wat in enige stadium tydens die vervaardigingsproses met grondstowwe of die onbeskermd produk in aanraking kom, gebruik word nie. Houttafels mag nie in die verwerkingsafdeling gebruik word nie. Die blaaie van voorbereidings- en verpakkingstafels moet gemaak word van gladde korrosiebestande materiaal wat waterdig is, en moet 'n higiëniese afwerking hê. Maklike verwyderbare snyplanke, met 'n higiëniese konstruksie en van ligkleurige materiaal (uitgesonderd hout), wat geskik is vir gebruik met voedsel, kan gebruik word. Die blaaie en tafels moet vinnige en doeltreffende dreinerings in die hand werk en moet glad en vry van barste en splete wees. Rottangmandjies in 'n goeie, skoon toestand kan by die aflaai van die vissersboot gebruik word.

3.1.2.2 Alle installasies, uitrusting en gereedskap moet skoon en in 'n goeie toestand gehou word. Skoonmaak- en ontsmettingsmiddels, warm en koue lopende water of versadigde stoom, waterslange, borsels en ander benodighede vir die skoonmaak van installasies, uitrusting en gereedskap moet beskikbaar wees. Installasies, uitrusting en gereedskap moet, nadat dit met 'n detergent of ander skoonmaakmiddel deeglik skoongemaak is, ontsmet word met 'n oplossing met 'n gehalte aan vry residuele chloor van minstens 20 d.p.m. of met 'n ander aanneemlike ontsmettingsoplossing. Die vrye residuele chloorkonsentrasie van chlooroplossings moet volgens die 5-sekonde-ortotolidienflitstoets of ander aanneemlike metode wat soortgelyke resultate lewer, bepaal word. Die uitrusting moet onmiddellik voor die aanvang van werksaamhede met water wat aan die vereistes van 3.1.4.1 of 3.1.4.2 voldoen, afgespoel word om die ontsmettingsmiddel te verwyder. Die hele verwerkingstelsel moet aan die einde van elke dag se werksaamhede skoongemaak word en dit moet onmiddellik voor verdere gebruik skoon wees. Handskoene en messe moet na gebruik of enige ander tyd wanneer sterilisering van uitrusting nodig is, deeglik skoongemaak en daarna met nat stoom, chloorwater of ander aanneemlike ontsmettingsoplossing of volgens 'n aanneemlike ontsmettingsproses ontsmet word. Vir hierdie doel moet steriliseringsgeriewe op gerieflike plekke beskikbaar wees.

3.1.2.3 Wanneer die houer vol of gedeeltelik vol grondstowwe of die produk is, mag dit nie op so 'n manier gestapel word dat die inhoud van een houer aan die onderkant van die houer hom raak nie. Houers mag nie regstreeks op die vloer gepak word nie.

3.1.1.14 An adequate number of wash basins or troughs with an acceptable soap or detergent, an acceptable disinfectant and an adequate supply of clean hot and cold running water or clean warm water for handwashing shall be provided at convenient points throughout the preparation or processing areas of the factory where the unprotected product is handled. Where hands are required to be dried after washing, disposable towels or other acceptable means of drying the hands shall be used.

3.1.1.15 The factory shall at all times be maintained in a hygienic state. Animals shall not be allowed in any part of the factory proper.

3.1.1.16 In relation to its handling, transportation, preparation, smoking, packing, freezing (where relevant) and storage, no operations shall be performed and no conditions shall be present that are detrimental to the product.

### 3.1.2 Equipment.

3.1.2.1 All plant, equipment and utensils coming into contact with the product shall be made of smooth-surfaced material resistant to corrosion by brine, shall be of hygienic design, and shall be so constructed as to facilitate their cleaning and sterilization and that of the areas beneath them. Lead and lead alloys other than solder shall not be used in the construction of equipment coming into contact with raw materials or the unprotected product at any stage in its manufacture. Wooden tables shall not be used in processing areas. The tops of preparation and packing tables shall be made of smooth corrosion-resistant material impervious to water, and shall have a hygienic finish. Easily removable cutting boards of hygienic construction made of acceptable light-coloured material other than wood and suitable for use with food, may be used. The tops of tables shall allow rapid and effective drainage and shall be smooth and free from cracks and crevices. Wicker baskets in good clean condition may be used in unloading the fishing vessel.

3.1.2.2 All plant, equipment and utensils shall be kept clean and in good repair. Cleaning and disinfecting materials, hot and cold running water or saturated steam, hose piping, brushes and other requisites necessary for the cleaning of plant, equipment and utensils shall be available. Plant, equipment and utensils after thorough cleaning with a detergent or other cleaning agent shall be disinfected by the application of a solution having a free residual chlorine content of at least 20 ppm, or other acceptable disinfecting solution. The free residual chlorine concentration of chlorinated solutions shall be determined by the orthotolidine 5 second "flash" test or other method giving equivalent results. Immediately before the commencement of operations, equipment shall be rinsed with water complying with the requirements of 3.1.4.1 or 3.1.4.2 to remove the disinfecting agent. The entire processing system shall be cleaned at the end of each day's operations, and shall be clean immediately before further use. Gloves and knives shall be thoroughly cleaned and then disinfected by the use of wet steam, chlorinated water or other acceptable disinfecting solution or procedure, after use, or at any time when sterilization of equipment is necessary. To this end sterilization facilities shall be available at convenient points.

3.1.2.3 Containers, when filled or partially filled with raw materials or with the product, shall not be stacked in a manner that allows contact of the contents of one container with the bottom of the container stacked above it. Containers shall not be stacked directly on the floors.

3.1.2.4 Gereedskap wat by die voorbereiding van die produk gebruik word, mag nie deur werknemers uit die fabriek verwyder word nie, behalwe vir herstelwerk of vervanging.

3.1.2.5 Toedraaimateriaal moet in korrosiebestande voorraadhouers met 'n higiëniese konstruksie gehou word.

3.1.2.6 Masjienonderdele en voorrade wat die produk kan besoedel, moet weg van die verwerkingsafdeling bewaar word.

3.1.3 *Bevriesings- en koelkamergeriewe*.—Vrieskamers, koelkamers en uitrusting moet doeltreffend werk en moet skoon en higiënies wees. Bewaarkamers vir die bevrore produk moet voorsien wees van outomatiese temperatuurregisterders met genoeg voelelemente om die totale lugtemperatuur binne die kamers op doeltreffende wyse te monitor. Geriewe wat deurlopende bevriesingsgeriewe verskaf, moet op dieselfde manier gemoniteer wees en alle registreerders moet die temperatuur noukeurig aandui. Lotbevriesingsfasiliteite moet van buitemeters of ander temperatuuraanwysers voorsien wees. Die temperatuur van koelkamers en bevriesingsgeriewe moet aangeteken word en 'n rekord daarvan moet vir ondersoek beskikbaar wees. Produkte mag nie regstreeks op die vloer of teenaan mure gepak word nie. Die produkte in lotbevriesingsfasiliteite, uitgesonderd plaatvriesingsfasiliteite, moet so gepak word dat die lugvloei tussen die stapels nie belemmer word nie. Daar moet aan die toepaslike vereistes van statutêre wette en regulasies voldoen word.

#### 3.1.4 Water.

3.1.4.1 *Drinkbare water*.—Behoudens die bepalings van 3.1.4.2, moet elke fabriek 'n toereikende voorraad skoon, drinkbare water hê wat vry is van stowwe in suspensie en bestanddele wat skadelik vir die produk of nadelig vir die gesondheid is. Die water moet d.m.v. uitvloeking, filtrering, chlorering of volgens 'n ander aanseemlike proses behandel wees om te verseker dat dit aan die volgende vereistes voldoen:

(a) In 95 persent van die watermonsters wat gedurende 'n jaar ondersoek is, mag daar geen kolivormige organismes in 100 ml wees nie.

(b) Geen monster mag *E. coli* in 100 ml bevat nie.

(c) Geen monster mag meer as 10 kolivormige organismes per 100 ml bevat nie.

(d) Kolivormige organismes mag nie in 100 ml van enige twee opeenvolgende monsters bespeur word nie.

Vir die doeleindes van waterontleding sluit die kolivormige groep alle gramnegatiewe, nie-spoorvormende stawe in wat laktose kan laat gis met die voortbring van suur en gas by 37 °C binne minder as 48 h. *E. coli* word geag gram-negatiewe, nie-spoorvormende stawe te wees wat laktose kan laat gis met die voortbring van suur en gas by 37 °C sowel as 44 °C binne minder as 48 h; dit produseer indool in peptonwater met triptofaan en kan nie natriumsitraat as enigste bron van koolstof benut nie.

Indien gechloreerde water die produk op enige wyse benadeel, moet sulke water onmiddellik voor gebruik ontchloor word.

3.1.4.2 *Seewater*.—Skoon, onbesoedelde, vars, lopende seewater kan in die fabriek gebruik word mits die telling kolivormorganismes nie 50 organismes per 100 ml water oorskry nie en mits geen *E. coli* I in 100 ml van die water bespeur kan word nie.

### 3.2 VEREISTES VIR WERKNEMERS BETROKKE BY DIE VOORBEREIDING EN VERWERKING VAN DIE PRODUK.

3.2.1. (a) Geen persoon wat aan 'n aansteeklike siekte ly of wat 'n draer is van 'n aansteeklike siekte, veral diaree, of wat aan 'n toestand ly wat etter of serum op

3.1.2.4 Utensils used in the preparation of the product shall not be removed from the factory by employees except for repair or replacement.

3.1.2.5 Wrapping materials shall be dispensed from corrosion-resistant containers of hygienic construction.

3.1.2.6 Spare parts for machinery and stores capable of contaminating the product shall be stored away from the processing area.

3.1.3 *Freezing and cold storage facilities*.—Freezers, cold stores and equipment shall operate effectively and shall be clean and hygienic. Storage rooms for the frozen product shall be equipped with automatic temperature recorders that have enough sensing elements to monitor effectively the overall air temperature inside the rooms. Continuous freezing facilities shall be similarly monitored, and all recorders shall accurately reflect the temperatures. Batch freezers shall be fitted with external gauges or other temperature indicators. A record of the temperatures of cold stores and freezing facilities shall be kept and shall be available for inspection. Products shall not be stacked directly on the floors or directly against the walls. The stacking of products in batch freezers other than plate freezers shall be such that air circulation between packages is not impeded. The applicable requirements of statutory acts and regulations shall be complied with.

#### 3.1.4 Water.

3.1.4.1 *Potable water*.—Subject to the provisions of 3.1.4.2, every factory shall have an adequate supply of clean potable water free from suspended matter and substances that are deleterious to the product or injurious to health. The water shall have been so treated by flocculation, filtration, chlorination or other acceptable process as to ensure compliance with the following requirements:

(a) Throughout any year, 95 per cent of samples shall not contain any coliform organisms in 100 ml.

(b) No sample shall contain *E. coli* in 100 ml.

(c) No sample shall contain more than 10 coliform organisms per 100 ml.

(d) Coliform organisms shall not be detectable in 100 ml of any two consecutive samples.

For the purpose of water analysis the coliform group shall include all gram-negative, non-spore-forming rods capable of fermenting lactose with the production of acid and gas at 37 °C in less than 48 h. *E. coli* shall be regarded as a gram-negative, non-spore-forming rod capable of fermenting lactose with the production of acid and gas at both 37 °C and 44 °C in less than 48 h; it produces indole in peptone water containing tryptophane and is incapable of utilizing sodium citrate as its sole source of carbon.

Where chlorinated water affects the product deleteriously in any way, such water shall be dechlorinated immediately before use.

3.1.4.2 *Sea water*.—Clean, uncontaminated, fresh running sea water may be used in the plant provided that the count of coliform organisms does not exceed 50 organisms per 100 ml of the water and no *E. coli* I are detectable in 100 ml of the water.

### 3.2 REQUIREMENTS FOR EMPLOYEES ENGAGED IN THE PREPARATION AND PROCESSING OF THE PRODUCT.

3.2.1 (a) No person who is suffering from or who is a carrier of any communicable disease, and diarrhoea in particular, or is suffering from any condition causing a

enige deel van die liggaam veroorsaak, mag toegelaat word om te help met die voorbereiding, verwerking, hantering of vervoer van die produk nie of om in enige deel van die fabriek waar die produk voorberei, verwerk, gehanteer of vervoer word, te werk nie.

(b) Geen persoon van wie dit bekend is dat hy aan 'n siekte ly wat deur voedsel oorgedra kan word of dat hy 'n draer is van so 'n siekte-infeksie of dat hy aan besmette wonde, sere of enige siekte ly, mag toegelaat word om in enige deel van die fabriek werk te doen wat dit moontlik maak dat hy die produk met patogeeniese organismes kan besmet nie.

(c) 'n Inspekteur het die reg om die bestuurder van enige fabriek te vra om enige mediese sertifikaat wat deur 'n werknemer ingedien is, aan hom vir ondersoek te toon.

(d) Geen persoon wat 'n snyplek het of aan 'n besering ly, mag met die voorbereiding, verwerking, hantering of vervoer van die produk in die fabriek help tensy die snyplek of besering so behandel of verbind is dat bloed nie die produk kan besoedel nie.

3.2.2 Nóg die werknemers se persoonlike besittings nóg hul voedsel mag in die voorbereidings-, verwerkings- of verpakkingsafdelings van die fabriek toegelaat word nie. Geen voedsel mag in hierdie afdelings deur personeel voorberei of genuttig word nie.

3.2.3 Spoeg en die gebruik van tabak in watter vorm ook al binne die voorbereidings-, verwerkings- en verpakkingsafdelings van die fabriek is verbode. Houers wat vir die voorbereiding of verpakking van die produk gebruik word, mag nie gebruik word om uit te drink nie.

3.2.4 Alle werknemers wat met die voorbereiding en verwerking van die produk tot en met die verpakkingstadium besig is, moet skoon, ligkleurige, beskermende klere en skoon, wasbare of wegdoenbare hoofbedekings om hulle hare te bedek, dra. Waterdigte, beskermende klere moet van plastiek of rubber wees. Oorpakke moet die werknemers se persoonlike klere heeltemal bedek. Moue mag nie tot onderkant die elmboog reik nie tensy dit deur plastiekoortrekmoue bedek is of in vries- of koelkamers gedra word. Alle beskermende klere moet van higiëniese ontwerp wees, moet heel gehou word en mag nie 'n bron van besoedeling vir die produk wees nie. Beskermende klere mag nie in werkkamers gebêre word nie; wanneer dit nie gebruik word nie, moet dit in kleedkamers gehou word en dit mag nie van die perseel af verwyder word nie, behalwe om in higiëniese toestande gewas te word. Waterdigte beskermende klere moet gedurende werkpouses en met besoeke aan sanitêre geriewe uitgetrek en aan hake by die uitgange van die voorbereidings- en verwerkingsafdelings gehang word.

3.2.5 Werknemers moet hulle vingernaels kort en skoon hou en moet hul hande voor hulle begin werk en na elke afwesigheid uit die verwerkingsafdeling van die fabriek met warm water en aanneemlike seep of detergent en dan met 'n aanneemlike ontsmettingsmiddel was, en daarna in skoon, lopende water afspoel. Naelverniss of -lak mag nie op vingernaels gebruik word nie en geen juwele mag gedra word nie.

3.3 BESOEKERS.—Enige persoon wat gedurende werksure die verpakkingsafdeling besoek of binnegaan, moet alle higiëniese vereistes nakom terwyl hy daar is en moet skoon beskermende klere dra wat die fabriek moet verskaf.

3.4 GERIEWE.—Voldoende kleedkamers, wasbakke, stortbaddens en sanitêre geriewe moet verskaf word. Daar moet genoeg warm en koue lopende water, skoon weggooihanddoeke of ander aanneemlike middel om die hande mee af te droog, naelborsels, toiletpapier en seep of detergent vir die werknemers beskikbaar wees.

discharge of pus or serum from any part of the body shall be allowed to engage in the preparation, processing, handling or transportation of the product, or to work in any part of the factory where the product is prepared, processed, handled or transported.

(b) No person known to be affected with a disease capable of being transmitted through food, or known to be a carrier of such disease infection, or afflicted with infected wounds, sores or any illness, shall be permitted to work in any part of the factory in a capacity in which there is a likelihood of such a person contaminating the product with pathogenic organisms.

(c) An inspector shall be entitled to demand that the manager of any factory shall produce for perusal by the inspector any medical certificate submitted to the manager by an employee.

(d) No person who is suffering from any cut or injury shall be engaged in any factory in the preparation, processing, handling or transportation of the product unless the cut or injury has been so treated or dressed that the discharge of blood on to the product has been prevented.

3.2.2 Neither workers' personal effects nor their food shall be present in the preparation, processing and packing area of the factory. No food shall be prepared or consumed by personnel in these areas.

3.2.3 Spitting and the use of tobacco in any form shall be prohibited within the preparation, processing and packing areas of the factory. Containers used in the preparation or in the packing of the product shall not be used for drinking purposes.

3.2.4 All employees engaged in the preparation and processing of the product up to and including the packing stage shall wear clean light-coloured protective clothing and clean washable or disposable headgear to cover their hair. Waterproof protective clothing shall be of plastic or rubber. Overalls shall cover the personal clothing of employees. Sleeves shall not extend below the elbow except when covered by plastic sleevelets or when worn in freezers and cold stores. All protective clothing shall be of hygienic design, shall be in good repair and shall not constitute a source of contamination of the product. Protective clothing shall not be stored in workrooms; when not in use it shall be kept in change-rooms and shall not be removed from the premises except for laundering under hygienic conditions. Waterproof protective clothing shall be taken off and suspended from wall pegs or hooks at exits from preparation and processing areas during intervals between work and during visits to sanitary conveniences.

3.2.5 Employees shall keep their finger nails short and clean and shall wash their hands with warm water and an acceptable soap or detergent and after than in an acceptable disinfectant and then rinse them in clean running water before commencing work and after each absence from the factory processing area. Varnish or lacquer shall not be used on finger-nails, and jewellery shall not be worn.

3.3 VISITORS.—Any person visiting or entering the packing area during the hours of operation shall observe all hygiene requirements when in that area, and shall wear clean protective clothing which shall be provided by the factory.

3.4 COMFORT FEATURES.—Adequate dressing rooms, wash-basins, shower baths and sanitary facilities shall be provided. Ample hot and cold running water, clean disposable towels or other acceptable means of drying the hands, nail brushes, toilet tissue and soap or detergent shall be available to employees.

#### 4. VEREISTES VIR BESTANDDELE.

4.1 TOESTAND VAN BESTANDDELE.—Alle grondstowwe en bestanddele wat by die voorbereiding van die produk gebruik word, moet skoon, gesond, van goeie kwaliteit en in elke opsig geskik vir menslike gebruik wees. Die bewaring, hantering en vervoer van vis vir verwerking ter see en op land moet in higiëniese toestande gedoen word.

#### 4.2 VOORBEREIDING VAN VIS.

4.2.1 Alle vis wat by die voorbereiding van die produk gebruik word, moet vry van skubbe wees en moet deeglik skoongemaak word in toestande wat verseker dat dit vry van besoedeling is. Binnegoed, derms en los stukkie vleis en vel moet verwyder word.

4.2.2 Indien die vis oopgesny word, moet dit netjies met 'n skerp mes gedoen word. Indien die vis ontgraat word, moet die vinne en ruggraat verwyder word. Oopgesnyde vis of vismootjies of dele van mootjies moet skoon wees en alle verflenterde rande moet afgesny word. Lelike bene wat uitsteek, moet verwyder word.

4.3 HOUTROOK.—Houtrook vir die rook van die produk moet verkry word deur aanneemlike hout wat vry is van gom, hars, verf, houtverduursamingsmiddels of ander bygevoegde stof, te verbrand.

4.4 KLEURSTOF.—Enige geskikte kleurstof wat in die regulasies opgestel kragtens die Wet op Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels toegelaat word, kan by die voorbereiding van die produk gebruik word.

4.5 SOUT.—Sout wat by die voorbereiding van die produk gebruik word, moet eetbaar wees en mag nie bitter wees nie. Dit moet vry wees van rooi halofielorganismes. Dit moet sover moontlik ook vry van ander halofielorganismes wees.

#### 5. VEREISTES VIR DIE PRODUK.

5.1 SOUT EN ROOK.—Die produk moet in higiëniese toestande aanneemlik gesout en gerook word en alle moontlike voorsorgmaatreëls moet getref word om te verseker dat die produk nie besoedel raak of bederf nie.

#### 5.2 ALGEMENE VEREISTES.

5.2.1 Die produk moet 'n aantreklike kenmerkende voorkoms en kleur hê. Die kleur moet vir bepaalde oppervlakke met en sonder vel eenvormig wees.

5.2.2 Die smaak en reuk van die produk moet kenmerkend wees. Dit moet vry wees van bysmake, byreuke, galsterige oliesmake en -reuke.

5.2.3 Daar mag geen stukkende stukkie vis aanwesig wees nie.

5.2.4 Die produk moet tydens die vervaardigingsproses vry gehou word van residuele gestolde bloed, afgeskuurde materiaal en swart spikkels as gevolg van die rookproses. Vel moet netjies gesny word en mag nie los of in so 'n mate beskadig wees dat dit wesenlik die voorkoms van die eenhede benadeel nie.

5.2.5 Die produk moet vry wees van vesels, sand, vuiligheid en ander vreemde stof.

5.2.6 Die produk mag geen teken van parasitiese of insekverpesting toon nie.

5.2.7 Preserveermiddels mag nie in die produk gebruik word nie.

5.2.8 Die temperatuur van die produk moet binne 6 h nadat dit uit die rookkamer gehaal is tot 4 °C verminder word. Indien dit nie vakuumpverpak gaan word nie, moet dit by 'n temperatuur van hoogstens 4 °C gehou word. Vakuumpverpakte produkte moet by 'n temperatuur van hoogstens -20 °C bewaar word.

#### 4. INGREDIENT REQUIREMENTS.

4.1 CONDITION OF INGREDIENTS.—All raw materials and ingredients used in the preparation of the product shall be clean, sound, of good quality, and in every way fit for human consumption. The storage, handling and transportation of fish for processing both at sea and on land shall be performed under hygienic conditions.

#### 4.2 PREPARATION OF FISH.

4.2.1 All fish used in the preparation of the product shall be free from scales and shall be cleaned thoroughly under conditions that ensure freedom from contamination. Viscera, gut and ragged pieces of flesh and skin shall be removed.

4.2.2 If the fish is split, it shall be smoothly cut with a sharp knife. If the fish is filleted, the fins and backbone shall be removed. Split fish or fillets or portions of fillets shall be clean and all ragged edges shall be trimmed off. Unsightly protruding bones shall be removed.

4.3 WOOD SMOKE.—Wood smoke for the smoking of the product shall be obtained by the use of acceptable wood that is free from gum, resin, paint, timber preservative or other added substance.

4.4 COLOURING SUBSTANCES.—Any suitable colouring substance permitted by the Regulations under the Foodstuffs, Cosmetics and Disinfectants Act, may be used in the preparation of the product.

4.5 SALT.—Salt used in the preparation of the product shall be edible and free from bitterness. It shall be free from red halophilic organisms. As far as possible it shall also be free from other halophilic organisms.

#### 5. REQUIREMENTS FOR THE PRODUCT.

5.1 SALTING AND SMOKING.—The product shall be acceptably salted and smoked under hygienic conditions, and every precaution shall be taken to ensure that the product does not suffer contamination or deterioration.

#### 5.2 GENERAL REQUIREMENTS.

5.2.1 The product shall have an attractive, characteristic appearance and colour, the latter uniform for particular surfaces with and without skin.

5.2.2 The flavour and odour of the product shall be characteristic. Off-flavours and off-odours and rancid oil flavours and odours shall not be present.

5.2.3 No broken pieces of fish shall be present.

5.2.4 In the manufacturing process the product shall be kept free of residual clotted blood, abraded material and black specks from the smoking process. Skin shall be neatly trimmed and not be loose or damaged to such an extent that it materially affects the appearance of the units.

5.2.5 The product shall be free from fibre, sand, grit and other extraneous matter.

5.2.6 The product shall show no evidence of parasitic or insect infestation.

5.2.7 Preservatives shall not be used in the product.

5.2.8 The temperature of the product shall be reduced to 4 °C within 6 h of its removal from the smoking chamber. If it is not to be vacuum-packed, it shall be maintained at a temperature not in excess of 4 °C. Vacuum-packed products shall be stored at a temperature not in excess of -20 °C.

**5.3 MIKROBIOLOGIESE VEREISTES.**—Volgens die metode in kolom 3 van Tabel 1 getoets, moet die produk aan die vereistes in kolom 2 voldoen.

TABEL I

1	2	3
Organisme	Gehalte, maks. per gram*	Toets-metode-onder-afdeling
Totale telling (by 35 °C) van lewensvatbare organismes.....	100 000	8,6
Halofielorganismes.....	1 000	8,14
<i>Staphylococcus aureus</i> .....	10	8,7
<i>Salmonella</i> .....	—	8,8 8,9
<i>Shigella</i> .....	—	8,10
Patogeniese klostridia.....	—	8,11
Koliforme organismes.....	10	8,12
<i>E. coli</i> 1.....	—	8,13

\* Die produk moet ook aan al die ander toepaslike vereistes wat kragtens die wet op Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels voorgeskryf is, voldoen.

## 6. VERPAKKING.

**6.1 VERPAKKING ONDER HIGIËNIESE TOESTANDE.**—Die produk moet onder toestande wat vryheid van besoedeling sal verseker, voorberei en in houters verpak word. Geen materiaal wat 'n smaak of reuk aan die produk verleen of enige verkleuring, bederf of besoedeling van die produk veroorsaak, mag as onmiddellike houer vir geroekte snoek gebruik word nie.

**6.2 VERPAKKING.**—Die produk moet verpak wees in doeltreffende buitehouers van hout, veselbord of ander soortgelyke aanneemlike materiaal. Houthouers moet gemaak wees van skoon droë hout wat geen tekens van skimmel toon nie. Die houters moet skoon, netjies en heel wees en mag geen reuk hê nie. Houters moet met vetdigte papier, sellulosefilm of nie-giftige inerte plastiek-film gevoer wees.

## 7. MERKE.

**7.1 MERKE OP HOUERS.**—Uitgesonderd soos in 7.4 toegelaat, moet die volgende besonderhede leesbaar en onuitwisbaar op elke houer aangebring wees in druk wat so groot en opvallend is soos voorgeskryf by regulasies kragtens die Wet op Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels en die Wet op Mate en Gewigte:

(a) Die naam en volledige besigheidsadres van die fabrikant, vervaardiger, eienaar of beherende maatskappij of, in die geval van houters wat vir enige ander persoon of organisasie verpak is, die naam en volledige besigheidsadres van sodanige persoon of organisasie;

(b) 'n juiste beskrywing van die produk;

(c) in die geval van produkte wat in die Republiek en die gebied Suidwes-Afrika verkoop gaan word, die netto massa van die inhoud;

(d) die land van herkoms;

(e) enige etikettering wat spesifiek volgens regulasie vereis word;

(f) die datum van vervaardiging en die identiteit van die fabriek waarin die produk verpak is; 'n kode kan hiervoor gebruik word mits die sleutel tot sodanige kode aan die owerheid wat hierdie spesifikasie toepas, bekendgemaak word.

## 7.2 ETIKETTE.

**7.2.1** Die besonderhede volgens 7.1 vereis, moet op die onmiddellike houer of op die toedraaipapier wat die onmiddellike houer bedek of op 'n etiket van aanneemlike materiaal wat aan die onmiddellike houer bevestig is of, in die geval van 'n deursigtige houer, in die onmiddellike houer ingesluit is, aangebring wees.

**5.3 MICROBIOLOGICAL REQUIREments.**—When tested in accordance with the method referred to in column 3 of Table 1, the product shall comply with the requirements given in column 2.

TABLE I

1	2	3
Organism	Content, max. per gram*	Test method subsection
Total count (at 35 °C) of viable organisms...	100 000	8,6
Halophilic organisms.....	1 000	8,14
<i>Staphylococcus aureus</i> .....	10	8,7
<i>Salmonella</i> .....	—	8,8 8,9
<i>Shigella</i> .....	—	8,10
Pathogenic clostridia.....	—	8,11
Coliform organisms.....	10	8,12
<i>E. coli</i> 1.....	—	8,13

\* The product shall also comply with all other applicable requirements promulgated in terms of the Foodstuffs, Cosmetics and Disinfectant Act.

## 6. PACKING.

### 6.1 PACKING UNDER HYGIENIC CONDITIONS.

—The product shall be prepared and packed into containers under conditions which ensure freedom from contamination. No material that imparts a flavour or an odour to the product or in any way causes discoloration, deterioration or contamination of the product shall be used as the immediate container for smoked snoek.

**6.2 PACKAGING.**—The product shall be packed in effective outer containers made of wood fibreboard, or similar acceptable material. Wooden containers shall be made of clean, dry wood showing no evidence of mould. The containers shall be clean, neat and unbroken and free from odour.

Containers shall be lined with grease-proof paper, cellulose film, or non-toxic, inert plastic film.

## 7. MARKING.

**7.1 MARKINGS ON CONTAINERS.**—Except as allowed for in terms of 7.4, the following information shall appear legibly and indelibly on each container in type of such size and presentation as are prescribed by regulation under the Foodstuffs, Cosmetics and Disinfectants Act and the Weights and Measures Act:

(a) The name and full business address of the manufacturer, producer, proprietor or controlling company or, in the case of containers packed for any other person or organisation, the name and full business address of that person or organisation;

(b) a true description of the product;

(c) in the case of products for sale in the Republic and in the territory of South-West Africa, the net mass of the contents;

(d) the country of origin;

(e) any labelling specifically called for by regulation;

(f) the date of manufacture and the identity of the factory in which the product was packed, the use of a code being permissible provided that the key to such code is disclosed to the authority administering this specification.

## 7.2 LABELS.

**7.2.1** The information required by 7.1 shall be printed on the immediate container or on the overwrap covering the immediate container, or on a label of acceptable material attached to the immediate container or, in the case of a transparent container, enclosed in the immediate container.

7.2.2 Etikette op houers moet skoon en netjies wees, en moet stewig bevestig wees. Dit mag nie oor ander etikette of oor besonderhede wat direk op die houer gedruk is, aangebring word nie. Slegs die fabrikant of sy gemagtigde agent mag die etikette aanbring.

7.2.3 Etiket- of verseëlingskleefmiddels wat onder die bewaringstoestande vir die verpakte produk maklik kan agteruitgaan, mag nie gebruik word nie.

7.3 MERKE OP VERPAKKINGS.—Buiteverpakking moet skoon, netjies en heel wees. Die getal en grootte of netto massa van die onmiddellike houers daarin moet saam met die besonderhede volgens 7.1 (a), (b), (e) en (f) vereis, behalwe dat die besigheidsadres nie volledig nie maar voldoende vir identifiseringsdoeleindes hoef te wees, op elke sodanige verpakking (krat, kartondoos, doos, ens.) in druk- of sjabloonletters aangebring wees.

7.4 MERKE OP HOUERS VIR UITVOER.—Houers vir uitvoer kan op 'n ander manier as volgens die vereistes van hierdie onderafdeling gemerk word, mits daar geen poging tot wanvoorstelling van die produk is nie en die besonderhede wat volgens 7.1 (f) verlang word, of op die houer of op 'n verpakkingstrokie wat bo-op die inhoud geplaas word voordat die houer verseël word, aangegee word. Daar moet aan die vereistes van die invoerland voldoen word.

## 8. METODES VIR MIKROBIOLOGIESE ONDER- SOEK.

8.1 ALGEMEEN.—Volg die aseptiese tegniek deur- gaans in die ondersoek.

8.2 GLASWARE.—Gebruik laboratorium- en maat- glasware van borosilikaatglas. So nie, kan glasware van sodaglas gebruik word mits dit geen swaar metale en geen vry alkali bevat nie. Indien toepaslik, kan plastiek- laboratoriumglas gebruik word mits dit nie-toksies vir batterië is.

- |                               |   |
|-------------------------------|---|
| (a) Toetsbuis.....            | Diameter van 35 mm en lengte van 200 mm.<br>Diameter van 10 mm en lengte van 100 mm.                    |
| (b) Petribakkies.....         | Diameter van 100 mm en hoogte van 20 mm.  |
| (c) Pipette.....              | Lewering van 1 ml (nie-gegradueer).<br>1 ml en 5 ml, tot by punt gradueer.<br>20 ml (vinnige lewering). |
| (d) Flesse.....               | Flesse met metaalskroefdroppe, inhouds-<br>vermoë 30 ml, 110 ml en 220 ml.                              |
| (e) Monsternemings-<br>flesse | Inhoudsvermoë van 250 ml, wybek met<br>proppe van geslypte glas.  |

Alle glas- en plastiekware wat by die mikrobiologiese ondersoek gebruik word, moet steriel wees. Maak alle houers met watte toe of verseël dit met geskikte sluitings nadat die glasware deeglik skoongemaak is. Steriliseer alle glasware, by voorkeur deur droë hitte by  $170 \pm 5^\circ \text{C}$  1 h lank aan te wend. So nie, indien dit nie uitvoerbaar is nie, bv. in die geval van glasware met rubbersluitings, hou dit 20 min lank in 'n outoklaaf by  $121 \pm 2^\circ \text{C}$ . (Hierdie temperatuur is gelykstaande met 'n druk van 103 kPa bo atmosferiese druk by seespieël, m.a.w. 207 kPa absoluut.) In die geval van plastiek- laboratoriumware, volg 'n aanneemlike steriliserings- metode.

### 8.3 KWECKBODEMS EN REAGENSE.

#### 8.3.1 Algemeen.

8.3.1.1 *Water*.—Al die water wat gebruik word, moet of glasgedistilleerde water of water van dieselfde suiwer- heid wees.

8.3.1.2 *Kwaliteit van bestanddele*.—Die kwaliteit van die bestanddele wat vir die voorbereiding van die kweckbodems en reagents gebruik word, moet aanneem- lik wees vir mikrobiologiese doeleindes<sup>(1)</sup>. Tensy anders vermeld, moet alle soute anhidries wees.

(1) Waar bestanddele van 'n spesifieke graad gespesifiseer word, kan besonderhede oor die verkryging daarvan van die Direkteur-generaal, Suid-Afrikaanse Buro vir Standarde, Private Bag X191, Pretoria verkry word.

7.2.2 Labels on containers shall be clean and neat and securely attached. They shall not be superimposed on other labels or on matter printed directly on the con- tainers. They shall not be applied by any person other than the manufacturer or his authorised agent.

7.2.3 Label or sealing adhesives that are liable to deteriorate under the conditions of storage of the packed product shall not be used.

7.3 MARKINGS ON PACKAGES.—Outer packages shall be clean, neat and unbroken, and on every such package (crate, carton, box, etc.) shall be printed or stencilled the number and size or net mass of the imme- diate containers in it and the information required by 7.1 (a), (b), (e) and (f), except that the business address need not be the full business address but must be suffi- cient for identification purposes.

7.4 MARKINGS ON CONTAINERS FOR EXPORT.—Containers for export may be marked differently to the requirements of this section provided that there is no attempt to misrepresent the product and that the informa- tion called for by 7.1 (f) appears either on the container or on a packing slip placed on the top of the contents before the container is sealed. The requirements of the importing country shall be complied with.

## 8. METHOS OF MICROBIOLOGICAL EXA- MINATION.

8.1 GENERAL.—Use aseptic technique throughout the examination.

8.2 GLASSWARE.—Use laboratory and volumetric glassware of borosilicate glass. Alternatively, glassware of soda glass may be used provided that it is free from heavy metals and free alkali. Plastics laboratory ware may be used where applicable provided that it is non-toxic to bacteria.

- |                       |   |
|-----------------------|---|
| (a) Test tubes.....   | 35 mm diameter × 200 mm long.<br>10 mm diameter × 100 mm long.                              |
| (b) Petri dishes..... | 100 mm diameter × 20 mm high.   |
| (c) Pipettes.....     | 1 ml delivery (non-graduated).<br>1 ml and 5 ml graduated to tip.<br>20 ml (fast delivery). |
| (d) Bottles.....      | Bottles with metal screw caps, capacity 30 ml,<br>110 ml and 220 ml.                        |
| (e) Sampling bottles  | 250 ml capacity, wide mouth with ground<br>glass stoppers.                                  |

All glassware and plastics ware used in the microbio- logical examination shall be sterile. After the glassware has been thoroughly cleaned, plug all vessels with cotton wool or seal them with suitable closures. Sterilize all glassware preferably by the application of dry heat at  $170 \pm 5^\circ \text{C}$  for 1 h. Alternatively, where this is not practicable, e.g. in the case of glassware fitted with rubber closures, autoclave at  $121 \pm 2^\circ \text{C}$  for 20 min. (This tem- perature corresponds to a pressure of 103 kPa above atmospheric pressure at sea level, i.e. 207 kPa absolute. In the case of plastics laboratory ware, sterilize by an acceptable method.

### 8.3 MEDIA AND REAGENTS.

#### 8.3.1 General.

8.3.1.1 *Water*.—All the water used shall be either glass- distilled water or water of equivalent purity.

8.3.1.2 *Quality of ingredients*.—The quality of the ingre- dients used in the preparation of the media and reagents shall be acceptable for microbiological purposes<sup>(1)</sup>. All salts are anhydrous unless otherwise stated.

(1) Where ingredients of special grade are specified, information regarding their sources of supply may be obtained from the Director General, South African Bureau of Standards, Private Bag X191, Pretoria.

8.3.1.3 *Noukeurigheid*.—Tensy anders aangewys, geld die volgende toleransies:

	<i>Toleransie, plus of minus</i>
(a) Op temperatuur.....	0,5 °C
(b) Op massa.....	1,0%
(c) Op volume.....	1,0%
(d) Op pH-waarde.....	0,1 pH-eenheid.

8.3.1.4 *Ontwaterde kweekbodems*.—Indien kweekbodems in ontwaterde vorm gebruik word, moet die fabrikant se aanwysings vir die aanmaak daarvan noukeurig gevolg word.

8.3.1.5 *Filtreer van kweekbodems*.—As dit nodig is om 'n kweekbodem by die voorbereiding daarvan te filtreer, gaan soos volg te werk:

(a) Filtreer kweekbodems wat geen stolmiddels bevat nie (m.a.w. vloeibare kweekbodems en boeljon) deur 'n medium vinnige filtreerpapier.

(b) Filtreer die kweekbodems wat stolmiddels bevat (bv. agar) deur 'n laag absorbeerwatte 10-15 mm dik, wat vooraf natgemaak is. Gebruik 'n trechter met 'n stoommantel om te voorkom dat die middel stol terwyl dit gefiltreer word. So nie, filtreer in 'n stoomkamer.

8.3.1.6 *Aansuiwering van pH-waarde van kweekbodems*.—Gebruik 'n 0,1-N-oplossing soutsuur of natriumhidroksied, soos toepaslik, tensy anders aangewys.

8.3.1.7 *Verdeling*.—Meet kweekbodems in die volumes uit en plaas by voorkeur in geskikte, steriele flesse met metaalskroefdroppe of in toetsbuis met watterproppe. Roet dit aanhoudend terwyl dit uitgemeet word. Gooi reagentse in reagentflesse.

8.3.1.8 *Sterilisering van die kweekbodem*.—Indien sterilisering in 'n outoklaaf gespesifiseer word, hou die kweekbodem 15 min lank by  $121 \pm 2$  °C, tensy anders aangewys. (Hierdie temperatuur is gelykstaande met 'n druk van 103 kPa bo atmosferiese druk by seespieël, d.w.s. 207 kPa absoluut.)

8.3.1.9 *Beheer van voorbereide kweekbodems*.—Sorg dat voorbereide kweekbodems die groei van die toepaslike organismes onder die gemelde broeitoestande kan onderhou.

8.3.1.10 *Bewaring*.—Sorg dat die voorbereide kweekbodems sorgvuldig teen blootstelling aan hitte en sonlig beskerm is. Tensy anders gespesifiseer, moet kweekbodems gebruik word binne 12 maande nadat dit voorberei is.

### 8.3.2 *Baird-Parker-agar*.

Bestanddele vir basale kweekbodem:

Agar.....	20,0 g
Glisien.....	12,0 g
Litiumchloried (gehidrateer).....	5,0 g
Vleisekstrak.....	5,0 g
Natriumpiruvaat.....	10,0 g
Triptoon.....	10,0 g
Gisekstrak.....	1,0 g
Sulfadimidienoplossing.....	25,0 ml

voorberei deur 0,5 g suiwer sulfadimidien in 25,0 ml 0,1-N-natriumhidroksiedoplossing op te los en met water tot 250 ml aan te vul.

Hierdie oplossing moet vars voorberei wees vir gebruik.

Los die bestanddele uitgesonderd die sulfadimidienoplossing in water op, voeg dan die sulfadimidienoplossing by en vul aan tot 1 liter. Laat dit kook sodat dit makliker oplos. Suiwer die pH-waarde tot 6,8-7,0 aan, meet in 90-ml-hoeveelhede in flesse uit sonder om dit te filtreer en steriliseer in 'n outoklaaf. Voeg die items wat aangegee word en wat alles vooraf deur filtrasie gesteriliseer en na sterilisasie in 'n koelkas gehou moet word en wat, in die geval van oplossing (a), binne 30 dae na

8.3.1.3 *Accuracy*.—Except where otherwise directed, the following tolerances shall apply:

	<i>Tolerance, plus or minus</i>
(a) On temperatures.....	0,5 °C
(b) On masses.....	1,0%
(c) On volumes.....	1,0%
(d) On pH values.....	0,1 pH unit.

8.3.1.4 *Dehydrated media*.—If dehydrated media are used, the manufacturers' instructions regarding their reconstitution shall be carefully followed.

8.3.1.5 *Filtration of media*.—Whenever it is necessary to filter a medium in the course of its preparation, proceed as follows:

(a) Filter media which do not contain solidifying agents (i.e. liquid media and broths) through a medium-speed filter paper.

(b) Filter those media that contain solidifying agents (e.g. agar) through a 10-15 mm thick layer of pre-wetted absorbent cotton wool. To prevent solidification of the medium during filtration, use a steam-jacketed funnel. Alternatively, carry out the filtration in a steam chamber.

8.3.1.6 *Adjustment of pH value of media*.—Unless otherwise directed, use a solution of approximately 0,1N of hydrochloric acid or of sodium hydroxide, as appropriate.

8.3.1.7 *Dispensing*.—Dispense media, in the volumes stated, preferably into suitable sterile metal screw-capped bottles or into cotton wool plugged test tubes. Stir constantly while dispensing. Dispense reagents into reagent bottles.

8.3.1.8 *Sterilization of the medium*.—When sterilization by autoclaving is specified, unless otherwise directed autoclave the medium at  $121 \pm 2$  °C for 15 min. (This temperature corresponds to a pressure of 103 kPa above atmospheric pressure at sea level, i.e. 207 kPa absolute.)

8.3.1.9 *Control of prepared media*.—Ensure that prepared media are capable of supporting the growth of the relevant organisms under the stated conditions of incubation.

8.3.1.10 *Storage*.—Ensure that prepared media are carefully protected from exposure to heat and sunlight. Unless otherwise specified, media shall be used within 12 months of preparation.

### 8.3.2 *Baird-Parker agar*.

Basal medium ingredients:

Agar.....	20,0 g
Glycine.....	12,0 g
Lithium chloride (hydrated).....	5,0 g
Meat extract.....	5,0 g
Sodium pyruvate.....	10,0 g
Tryptone.....	10,0 g
Yeast extract.....	1,0 g
Sulphadimidine solution.....	25,0 ml

Prepared by dissolving 0,5 g of pure sulphadimidine in 25,0 ml of 0,1N sodium hydroxide solution and making up to 250 ml with water.

This solution must be freshly prepared for use.

Dissolve the ingredients other than the sulphadimidine solution in water and then add the sulphadimidine solution and make up to 1 litre. Aid solution by boiling. Adjust the pH value to 6,8-7,0, dispense without filtration 90 ml volumes into bottles, and sterilize by autoclaving. Before pouring plates, to each 90 ml of the basal medium at 45-50 °C add the items listed, sterilized in

voorbereiding gebruik moet word, by elke 90-ml-hoeveelheid van die basale kweekbodem by 45-50 °C voordat plate gegiet word:

- (a) 1% (m/v)-kaliumtelluriëtoplossing..... 1,0 ml  
(b) Eiergeleemulsie..... 5,0 ml

Meng deeglik en giet plate; gebruik ongeveer 15 ml per plaat. Gebruik die plate binne 24 h nadat dit gegiet is. Droog die oppervlak van die plate minstens 1 h lank by 45 °C voordat dit gebruik word.

Berei die eiergeleemulsie soos volg voor:

Skei die geel van die wit en voeg water by in die verhouding van 4 volumes water tot 1 volume eiergeel. Meng deeglik en verhit 2 h lank in 'n waterbad by 45 °C. Sentrifugeer om die neerslag te verwyder of laat die mengsel oornag in 'n koelkas staan. Gooi die bofloei-stof af en steriliseer deur dit te filtreer. Gooi 5-ml-hoeveelhede in steriele flesses uit en bewaar dit in 'n koelkas.

### 8.3.3 *Differensiële versterkte klostridiumkweekbodem (dubbele sterkte).*

Bestanddele vir basale kweekbodem:

1-sisteien.....	0,5 g
Glukose.....	1 g
Vleisekstrak.....	10 g
Peptoon.....	10 g
Natriumasetaat trihidraat.....	5 g
Oplosbare stysel.....	1 g
Gisekstrak.....	1,5 g

Voeg die peptoon, vleisekstrak, natriumasetaat en gisekstrak by 400 ml water. Berei 'n flodder van die stysel in koue water, voeg dit by 80 ml kookwater en vul dit tot 100 ml aan. Voeg hierdie styseloplossing by die eerste mengsel, stoom dit 30 min lank om al die bestanddele op te los en voeg die glukose en 1-sisteien (wat maklik oplos) dan by. Suiwer die pH-waarde tot 7,1-7,2 aan en filtreer deur papierpulp terwyl dit nog warm is. Meet 12,5-ml-hoeveelhede van hierdie basale kweekbodem in flesses uit en steriliseer in 'n outoklaaf. Stoom die kweekbodem ongeveer 10 min lank op die dag waarop dit gebruik gaan word, laat dit tot by 50 °C afkoel en voeg 0,25 ml van elk van die volgende by elke fles wat die kweekbodem bevat:

- (a) 4 persent (m/v)-natriumsulfietoplossing.  
(b) 7 persent (m/v)-ferrisitraatskaaloplossing.

By die voorbereiding van oplossing (b), help die oplossing van die ferrisitraatskaal aan deur dit ongeveer 5 min lank te verhit en dan af te koel. Steriliseer beide oplossings (a) en (b) deur dit te filtreer en bewaar dit in flesses by 3-5 °C. Berei elke 14 dae vars oplossings voor.

### 8.3.4 *Kovac reagens.*

Bestanddele:

Amielalkohol (piridienvry).....	75 ml
Soutsuur, gekonsentreer.....	25 ml
p-dimetielaminobenzaldehyd.....	5 g

Los die aldehid in die alkohol op en help oplossing aan deur dit in 'n waterbad stadig tot by 50-55 °C te verhit. Koel af en voeg die suur by. Beskerm teen lig en bewaar in 'n bruin fles by ongeveer 4 °C. Die reagens moet liggeel van kleur wees. (Party soorte amielalkohol gee die reagens 'n baie donker kleur en maak dit onaanneemlik.) Laat 24 h lank staan voordat dit gebruik word.

### 8.3.5 *Laktoseboeljon.*

Bestanddele:

Laktose.....	5 g
Vleisekstrak.....	3 g
Peptoon.....	5 g

Los die bestanddele in water op en vul aan tot 1 liter. Suiwer die pH-waarde tot 6,7 aan, meet 200-ml-hoeveelhede in flesses uit en steriliseer in 'n outoklaaf.

advance by filtration, stored in a refrigerator after sterilization and, in the case of solution (a), used within 30 d of preparation:

- (a) 1 per cent (m/v) solution of potassium tellurite.... 1,0 ml  
(b) Egg yolk emulsion..... 5,0 ml

Mix well and pour plates, using about 15 ml per plate. Use the plates within 24 h of pouring. Dry the surface of the plates for at least 1 h at 45 °C before use.

Prepare the egg yolk emulsion as follows:

Separate yolks from whites and add water in the ratio of 4 volumes of water to 1 volume of egg yolk. Mix thoroughly and heat in a water bath at 45 °C for 2 h. Centrifuge to remove the precipitate, or allow the mixture to stand overnight in a refrigerator. Decant the supernatant fluid and sterilize it by filtration. Dispense 5 ml volumes into sterile bottles and store in a refrigerator.

### 8.3.3 *Differential reinforced clostridium medium (double strength).*

Basal medium ingredients:

1-cysteine.....	0,5 g
Glucose.....	1 g
Meat extract.....	10 g
Peptone.....	10 g
Sodium acetate trihydrate.....	5 g
Soluble starch.....	1 g
Yeast extract.....	1,5 g

To 400 ml of water add the peptone, meat extract, sodium acetate and yeast extract. Prepare a slurry of the starch in cold water, add to about 80 ml of boiling water and make up to 100 ml.

Add this starch solution to the first mixture, steam for 30 min to dissolve all the ingredients, and then add the glucose and 1-cysteine (which dissolve readily). Adjust the pH value to 7,1-7,2 and filter while hot through paper pulp.

Dispense 12,5 ml volumes of this basil medium into bottles and sterilise by autoclaving. On the day that the medium is to be used, steam it for approximately 10 min, allow to cool to 50 °C, and add 0,25 ml of each of the following to each bottle of the medium:

- (a) 4 per cent (m/v) solution of sodium sulphite.  
(b) 7 per cent (m/v) solution of ferric citrate scales.

When solution (b) is prepared, aid solution of the ferric citrate scales by heating for about 5 min, and then cool. Sterilize both solutions (a) and (b) by filtration and store in bottles at 3-5 °C. Prepare fresh solutions every 14 days.

### 8.3.4 *Kovacs reagent.*

Ingredients:

Amyl alcohol (pyridine free).....	75 ml
Hydrochloric acid, concentrated.....	25 ml
p-Dimethylaminobenzaldehyde.....	5 g

Dissolve the aldehyde in the alcohol, aiding solution by warming gently to 50-55 °C in a water bath. Cool and add the acid. Protect from light and store in a brown bottle at about 4 °C. The reagent must be light yellow in colour. (Some brands of amyl alcohol cause the reagent to have a very dark colour and to be unsatisfactory.) Allow to stand for 24 h before use.

### 8.3.5 *Lactose broth.*

Ingredients:

Lactose.....	5 g
Meat extract.....	3 g
Peptone.....	5 g

Dissolve the ingredients in water and make up to 1 litre. Adjust the pH value to 6,7, dispense 200 ml volumes into bottles, and sterilize by autoclaving.

### 8.3.6 MacConkey-agar.

#### Bestanddele:

Agar.....	15 g
Galsout No. 3.....	1,5 g
Kristalviolet.....	0,002 g
Laktose.....	10 g
Neutraalrooi.....	0,03 g
Pepton.....	20 g
Natriumchloried.....	5 g

Los die bestanddele in water op en vul aan tot 1 liter. Help oplossing aan deur dit te kook. Suiwer die pH-waarde aan tot 7,4 en filtreer terwyl dit warm is. Meet 15-ml-hoeveelhede in flesse uit. Steriliseer in 'n outoklaaf.

### 8.3.7 MacConkey-boeljon.

#### Bestanddele:

Galsout.....	5 g
Broomkresolpers.....	0,01 g
Laktose.....	10 g
Pepton.....	20 g
Natriumchloried.....	5 g

Los die bestanddele in water op en vul aan tot 1 liter. Suiwer die pH-waarde tot 7,4 aan. Filtreer, meet uit in flesse wat omgekeerde Durham-buise bevat en steriliseer in 'n outoklaaf.

### 8.3.8 Gemodifiseerde soutwatervoedingsagar.

#### Bestanddele:

Ferrichloried, gehidrateer ( $\text{FeCl}_3 \cdot 7\text{H}_2\text{O}$ ).....	0,025 g
Gliserol.....	10 g
Magnesiumnitraat, gehidrateer ( $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ).....	1 g
Magnesiumsulfaat, gehidrateer ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ).....	5 g
Proteosepepton No. 3.....	5 g
Natriumchloried.....	200 g

Los die bestanddele in water op en vul aan tot 1 liter. Suiwer die finale pH-waarde aan tot 7,2-7,5.

Deel die oplossing in twee dele. Voeg 3 persent agar by een deel. Steriliseer die dele afsonderlik in 'n outoklaaf. Smelt die deel wat die agar bevat en laat dit tot by ongeveer 50 °C afkoel voor gebruik. Verhit die ander deel tot ongeveer 50 °C, voeg 10 persent steriele afgeroomde melk by en meng deeglik. Meng die twee dele in 'n fles en meet hoeveelhede van ongeveer 20 ml uit met 'n pipet met vinnige lewering.

### 8.3.9 Gewone Konynplasma.

### 8.3.10 Voedingsboeljon.

#### Bestanddele:

Vleisekstrak.....	10 g
Pepton.....	10 g
Natriumchloried.....	5 g

Los die bestanddele in water op en vul aan tot 1 liter. Help oplossing aan deur dit te kook. Suiwer die pH-waarde tot 7,5 aan en filtreer. Meet 10-ml-hoeveelhede in flesse uit en steriliseer in 'n outoklaaf.

**8.3.11 Peptonwaterverdunner.**—Los 1 g pepton in 1 liter water op. Suiwer die pH-waarde tot 7,1 aan. Meet 9-ml- en 100-ml-hoeveelhede in flesse uit. Steriliseer in 'n outoklaaf.

### 8.3.12 Plaattellingagar.

#### Bestanddele:

Agar.....	15 g
Glukose.....	1 g
Tripton.....	5 g
Gisekstrak.....	2,5 g

Los die bestanddele in water op en vul aan tot 1 liter. Suiwer die pH-waarde tot 7,2 aan. Meet 15-ml-hoeveelhede in flesse uit. Steriliseer in 'n outoklaaf.

### 8.3.6 MacConkey agar.

#### Ingredients:

Agar.....	15 g
Bile salts No. 3.....	1,5 g
Crystal violet.....	0,002 g
Lactose.....	10 g
Neutral red.....	0,03 g
Peptone.....	20 g
Sodium chloride.....	5 g

Dissolve the ingredients in water and make up to 1 litre. Aid solution by boiling. Adjust the pH value to 7,4 and filter while hot. Dispense 15 ml volumes into bottles. Sterilize by autoclaving.

### 8.3.7 MacConkey broth.

#### Ingredients:

Bile salts.....	5 g
Bromocresol purple.....	0,01 g
Lactose.....	10 g
Peptone.....	20 g
Sodium chloride.....	5 g

Dissolve the ingredients in water and make up to 1 litre. Adjust the pH value to 7,4, filter, dispense 10 ml volumes into bottles containing inverted Durham tubes, and sterilize by autoclaving.

### 8.3.8 Modified nutrient brine agar.

#### Ingredients:

Ferric chloride, hydrated ( $\text{FeCl}_3 \cdot 7\text{H}_2\text{O}$ ).....	0,025 g
Glycerol.....	10 g
Magnesium nitrate, hydrated [ $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ].....	1 g
Magnesium sulphate, hydrated ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ).....	5 g
Proteose peptone No. 3.....	5 g
Sodium chloride.....	200 g

Dissolve the ingredients in water and make up to 1 litre.

Adjust the final pH value to 7,2-7,5.

Divide the solution into two portions. To one portion add 3 per cent of agar. Sterilize the portions separately by autoclaving. Before use melt the portion containing the agar and cool to about 50 °C. Warm the other portion to about 50 °C, add 10 per cent of sterile skim milk, and mix well. Mix the two portions together in a flask and with a fast delivery pipette dispense about 20 ml volumes.

### 8.3.9 Normal rabbit plasma.

### 8.3.10 Nutrient broth.

#### Ingredients:

Meat extract.....	10 g
Peptone.....	10 g
Sodium chloride.....	5 g

Dissolve the ingredients in water and make up to 1 litre. Aid solution by boiling. Adjust the pH value to 7,5 and filter. Dispense 10 ml volumes into bottles and sterilize by autoclaving.

**8.3.11 Peptone water diluent.**—Dissolve 1 g of peptone in 1 litre of water. Adjust the pH value to 7,1. Dispense 9 ml and 100 ml volumes into bottles. Sterilize by autoclaving.

### 8.3.12 Plate-Count agar.

#### Ingredients:

Agar.....	15 g
Glucose.....	1 g
Tryptone.....	5 g
Yeast extract.....	2,5 g

Dissolve the ingredients in water and make up to 1 litre. Adjust the pH value to 7,2. Dispense 15 ml volumes into bottles. Sterilize by autoclaving.

### 8.3.13 *Versterkte klostridiumagar.*

#### Bestanddele:

Agar.....	15 g
1-sisteien.....	0,5 g
Glukose.....	5 g
Vleisekstrak.....	10 g
Peptoon.....	10 g
Natriumasetaattrihiidraat.....	5 g
Oplosbare stysel.....	1 g
Gisekstrak.....	3 g

Los die bestanddele in water op en vul aan tot 1 liter. Help oplossing aan deur dit te stoom. Filtreer deur papierpulp, suiwer die pH-waarde tot 7,4 aan, meet 15-ml-hoeveelhede in flesse uit en steriliseer in 'n outoklaaf.

### 8.3.14 *Selenietboeljon.*

#### Bestanddele:

Natriumseleniet.....	4 g
Dinatriumfosfaat ( $\text{Na}_2\text{HPO}_4$ ).....	10 g
Laktose.....	4 g
Peptoon.....	5 g

Los die peptoon, laktose en dinatriumfosfaat in 750 ml water op en help oplossing aan deur stadig te verhit; steriliseer in 'n outoklaaf. Los die seleniet in 250 ml koue water op, steriliseer deur dit te filtreer en voeg die steriele filtraat by die gesteriliseerde peptoon-laktose-fosfaat-oplossing. Meng deeglik en meet 10-ml-hoeveelhede in flesse uit. Moenie die kweekbodem verder verhit nie. Indien dit nie gebruik word op die dag waarop dit voorberei is nie, bewaar by 4 °C.

### 8.3.15 *SS-agar.*

#### Bestanddele:

Agar.....	15 g
Galsout No. 3.....	8,5 g
Briljante groen.....	0,000 33 g
Ferrisitraat.....	1 g
Laktose.....	10 g
Vleisekstrak.....	5 g
Neutraalrooi.....	0,025 g
Peptoon.....	10 g
Natriumsitraat.....	10 g
Natriumtiosulfaat.....	8,5 g

Los die bestanddele in water op en vul aan tot 1 liter. Help oplossing aan deur dit te kook. Roer om verbranding te voorkom. Suiwer die pH-waarde tot 7,0 aan. Moenie in 'n outoklaaf plaas nie. Verkoel tot by ongeveer 50 °C en gooi hoeveelhede van ongeveer 15 ml in petribakkies uit.

**8.3.16 *Natriumchloriedoplossing.***—Los 9 g natriumchloried in 1 liter water op. Meet 10-ml-hoeveelhede in flesse uit. Steriliseer in 'n outoklaaf.

### 8.3.17 *Tryptoonwater.*

#### Bestanddele:

Tryptoon.....	10 g
Natriumchloried.....	5 g

Los die bestanddele in water op en vul aan tot 1 liter. Suiwer die pH-waarde tot 7,5 aan. Meet 9-ml-hoeveelhede in flesse uit. Steriliseer in 'n outoklaaf.

**8.4 VOORBEREIDING VAN MONSTERS.**—Gebruik 'n steriele ontleedmes en 'n tangetjie om ongeveer 20 g van die monster te verwyder en om dit na 'n vooraf geweegde monsternemingsfles oor te plaas. Voeg genoeg peptoonwaterverdunner (8.3.11) by om 'n 1:10 (m/v)-verspreiding te gee en masereer lank genoeg om 'n totale getal van 15 000 tot 20 000 omwentelinge van die mascerlemme te gee, maar dit moet in geen geval langer as 2,5 min wees nie. Gebruik die 1:10-verspreiding van die monster wat so verkry is vir die toetse in 8.6, 8.7, 8.11, 8.12 en 8.14.

**8.5 GIET VAN PLATE.**—Wanneer dit ook al nodig is om plate, soos by teltegnieke, te giet, pipetteer die inokulum cers in die plaat (petribakkie), voeg dan die gesmelte en getemperde kweekbodem by en meng. Sorg dat geen deel van die inhoud van die bakkie

### 8.3.13 *Reinforced clostridium agar.*

#### Ingredients:

Agar.....	15 g
1-cysteine.....	0,5 g
Glucose.....	5 g
Meat extract.....	10 g
Peptone.....	10 g
Sodium acetate trihydrate.....	5 g
Soluble starch.....	1 g
Yeast extract.....	3 g

Dissolve the ingredients in water and make up to 1 litre. Aid solution by steaming. Filter through paper pulp, adjust the pH value to 7,4, dispense 15 ml volumes into bottles, and sterilize by autoclaving.

### 8.3.14 *Selenite broth.*

#### Ingredients:

Sodium selenite.....	4 g
Disodium phosphate ( $\text{Na}_2\text{HPO}_4$ ).....	10 g
Lactose.....	4 g
Peptone.....	5 g

Dissolve the peptone, lactose and disodium phosphate in 750 ml of water, aiding solution by gentle heating, and sterilize by autoclaving. Dissolve the selenite in 250 ml of cold water, sterilize by filtration, and add the sterile filtrate to the sterilized peptone-lactose-phosphate solution. Mix well and dispense 10 ml volumes into bottles. Do not heat the medium further. If not used on the day of preparation, store at 4 °C.

### 8.3.15 *SS agar.*

#### Ingredients:

Agar.....	15 g
Bile salts No. 3.....	8,5 g
Brilliant green.....	0,000 33 g
Ferric citrate.....	1 g
Lactose.....	10 g
Meat extract.....	5 g
Neutral red.....	0,025 g
Peptone.....	5 g
Sodium citrate.....	10 g
Sodium thiosulphate.....	8,5 g

Dissolve the ingredients in water and make up to 1 litre. Aid solution by bringing to the boil. Agitate to prevent charring. Adjust the pH value to 7,0. Do not autoclave. Cool to about 50 °C and pour about 15 ml volumes into petri dishes.

**8.3.16 *Sodium chloride solution.***—Dissolve 9 g of sodium chloride in 1 litre of water. Dispense 10 ml volumes into bottles. Sterilize by autoclaving.

### 8.3.17 *Tryptone water.*

#### Ingredients:

Tryptone.....	10 g
Sodium chloride.....	5 g

Dissolve the ingredients in water and make up to 1 litre. Adjust the pH value to 7,5. Dispense 9 ml volumes into bottles. Sterilize by autoclaving.

**8.4 PREPARATION OF SAMPLES.**—Using a sterile scalpel and forceps, remove approximately 20 g of sample and transfer to a previously tared sampling bottle. Add enough peptone water diluent (8.3.11) to give a 1:10 (m/v) dispersion, and macerate for sufficient time to give a total number of 15 000 to 20 000 revolutions of the macerator blades, but in no case for longer than 2,5 min. Use the 1:10 dispersion of the sample so obtained for the tests described in 8.6, 8.7, 8.11, 8.12 and 8.14.

**8.5 POURING OF PLATES.**—Whenever it is necessary to pour plates as in counting techniques, first pipette the inoculum into the plate (petri dish), then add the melted and tempered medium, and mix. Avoid spilling

gedurende hierdie proses uitstort nie. Die beste manier om dit te doen is om die bakkie op 'n tafelblad te plaas en die inhoud stadig te werwel deur die bak horisontaal in die rondte te draai.

**8.6 TOTALE TELLING LEWENSVATBARE ORGANISMES.**—Berei reeksverdunnings uit die 1:10-verspreiding van die monster (8.4) voor deur 1 ml van die verspreiding in 'n fles van 9 ml peptonwaterverdunner (8.3.11) te pipetteer en deur verdere verdunnings te maak deur 1 ml van die eerste verdunning in 'n ander fles met 9 ml peptonwaterverdunner te pipetteer. Herhaal hierdie proses, elke keer met 'n skoon pipet, totdat 'n verdunning van 1 monsterdeel op 10 000 dele peptonwaterverdunner verkry word. Meng die inhoud van elke fles deeglik voordat 'n verdere verdunning daarvan voorberei word. Neem uit elke verdunning van die monster twee 1,0-ml-hoeveelhede en plaas elke hoeveelheid oor in 'n plaat (petriblaai). Neem vir elke plaat een 15-ml-hoeveelheid plaattellingagar (8.3.12), smelt en temper dan tot 45 °C. Giet die plate (8.5). Laat die agar stol, keer die plate om, plaas hulle (met geskikte etikette) oor na 'n inkubator en inkubeer 48 h lank by 35 °C.

Sorg dat hoogstens 30 min altesaam tussen die voorbereiding van die verdunnings van die monster en die finale giet van die plate verloop.

Haal die plate na inkubasie uit die inkubator en tel die kolonies op dié plate wat tussen 30 en 300 kolonies bevat. Teken die resultate aan. Bereken die totale telling lewensvatbare organismes deur die gemiddelde te verkry van die afsonderlike tellings op elke plaat met 'n bepaalde verdunning en hierdie gemiddelde telling met die betrokke verdunningsfaktor te vermenigvuldig (bv. in die geval van 'n 1:1 000-verdunning, vermenigvuldig die gemiddelde platetelling met 1 000).

### 8.7 STAPHYLOCOCCUS AUREUS.

**8.7.1 Isolering.**—Pipetteer 1,0 ml van die 1:10-verspreiding van die monster (8.4) op elk van die 5 plate met Baird-Parker-agar (8.3.2) wat vooraf gegiet, afgekoel en gedroog is. Smeer die inokulum met 'n steriele glas- of draadoog oor die oppervlak van die plaat en laat dit goed in die agar wegtrek. Inkubeer die plate 24-48 h lank by 37 °C en ondersoek hulle dan.

Koagulasepositiewe kolonies van *Staphylococcus aureus* word aangetref as klein (diameter 1,0-1,5 mm) konvekse, blink kolonies met 'n swart kern en 'n smal, wit, ononderbroke rand, wat omring word deur 'n helder sone wat 2-5 mm diep in die ondeurskynende kweekbodem strek. Alhoewel sulke kolonies visueel uitgeken kan word, staaf die uitkenning deur die koagulasetoets (kyk 8.7.2) uit te voer. Die telling staphylococci per gram = 2 x die totale getal sulke kolonies op al vyf plate.

**8.7.2 Koagulasetoets.**—Haal vermoede staphylococcus-kolonies van die Baird-Parker-agarplate (8.7.1) af en plaas dit oor na flesses voedingsboeljon (8.3.10). Inkubeer 18-24 h lank by 37 °C. Verdun die konynglas (8.3.9) 1:10 met natriumchloriedoplossing (8.3.16). Plaas 0,5 ml verdunde plasma in 'n klein proefbuisie en voeg 5 druppels van die voedingsboeljonkultuur van die organisme by. Inkubeer die buis by 37 °C. Ondersoek na 1 h en met tussenpose tot 24 h vir die aanweishgied van fibrinstolsel wat 'n positiewe resultaat sal aandui. 'n Stolsel kan na vorming teen 'n veranderende tempo geliseer word, sodat mens versigtig moet wees om seker te maak dat 'n verkeerde negatiewe lesing nie verkry word nie. Dit is raadsaam om in enige reeks toetse, buise wat staphylococcus-stamme bevat wat as (a) koagulasepositief en (b) koagulase-negatief bekend is, as kontrole in te sluit.

any of the contents of the dish during this process. This is best achieved by placing the dish on a table top and gently swirling the contents by moving the dish in a circle in the horizontal plane.

**8.6 TOTAL COUNT OF VIABLE ORGANISMS.**—Prepare serial dilutions from the 1:10 dispersion of the sample (8.4) by pipetting 1 ml of this dispersion into a bottle containing 9 ml of peptone water diluent (8.3.11), making further dilutions by pipetting 1 ml of the first dilution into another bottle containing 9 ml of peptone water diluent, and repeating this procedure, using a fresh pipette each time, until a dilution of 1 part sample in 10 000 volumes of peptone water diluent is obtained. Mix each bottle thoroughly before preparing a further dilution from it. From each dilution of the sample take two 1,0 ml volumes, and transfer each volume to a plate (petri dish). For each plate take one 15 ml volume of plate-count agar (8.3.12), melt, and then temper it to 45 °C. Pour the plates (8.5). Allow the agar to set, invert the plates, transfer them (suitably labelled) to an incubator, and incubate at 35 °C for 48 h.

Ensure that the total period between the preparation of the dilutions of the sample and the final plating does not exceed 30 min.

After incubation, remove the plates from the incubator, and count the colonies on those plates that contain between 30 and 300 colonies. Record these results. Calculate the total count of viable organisms by averaging the individual counts on each plate of a given dilution and multiplying this average count by the dilution factor involved (e.g. for a dilution of 1:1 000 multiply the average plate count by 1 000).

### 8.7 STAPHYLOCOCCUS AUREUS.

**8.7.1 Isolation.**—On to each of 5 previously poured, cooled and dried plates of Baird-Parker agar (8.3.2) pipette 1,0 ml of the 1:10 dispersion of the sample (8.4). Spread the inoculum over the surface of the plate with a sterile glass or wire spreader, and allow it to soak well into the agar. Incubate the plates at 37 °C for 24-48 h and then examine them.

Coagulase-positive colonies of *Staphylococcus aureus* show as small (1,0-1,5 mm diameter) black-centred colonies that are convex and shiny and have a narrow white unbroken margin, surrounded by a clear zone that extends 2-5 mm into the opaque medium.

Although such colonies are identifiable visually, confirm identification by using the coagulase test (see 8.7.2). The count of staphylococci per gram = 2 x the total number of such colonies on all 5 plates.

**8.7.2 Coagulase test.**—Pick off suspected staphylococcal colonies from the Baird-Parker agar plates (8.7.1) and transfer them into bottles of nutrient broth (8.3.10). Incubate at 37 °C for 18-24 h. Dilute rabbit plasma (8.3.9) 1:10 with sodium chloride solution (8.3.16). Place 0,5 ml of diluted plasma in a small test tube and add 5 drops of the nutrient broth culture of the organism. Incubate the tube at 37 °C. Examine after 1 h and at intervals up to 24 h for the presence of a fibrin clot which constitutes a positive result. A clot may, after forming, be lysed at a variable rate so that care must be taken to avoid a false negative reading. In any batch of tests it is advisable to include, as controls, tubes containing strains of staphylococcus known to be (a) coagulase positive, and (b) coagulase negative.

### 8.8 SALMONELLA.

(a) Plaas 20 g van die monster (8.4) oor na 'n vooraf geweegde monsternemingsfles. Voeg genoeg laktoseboeljon (8.3.5) by om 'n 1:10(m/v)-verspreiding te gee. Masereer soos in 8.4 en inkubeer 6 h lank by 37 °C. Gebruik 'n platinaoogdraadjie met binnediamter van 4 mm om twee oëvol van dié kultuur na 10 ml selenietboeljon (8.3.14) oor te plaas en inkubeer dit 18-24 h lank by 43 °C. Smeer een oogvol van die kultuur op so 'n wyse oor 'n plaat SS-agar (8.3.15) dat dit die groei van afsonderlike kolonies aanwakker. Inkubeer die plaat 18-24 h lank by 37 °C.

(b) Onderzoek die plaat vir vermoede kolonies *Salmonella spp*, wat kleurloos voorkom.

(c) Stel die suiwerheid van die kultuur vas deur 10 vermoede *Salmonella*-kolonies of al sulke kolonies (water ook al die minste is) uit te soek, elke kolonie oor die oppervlak van 'n MacConkey-agarplaat (8.3.6) te smeer, dit 18-24 h lank by 37 °C te inkubeer en die identiteit van die organismes volgens aanneemlike biochemiese en serologiese tegnieke te bevestig.

### 8.9 SALMONELLA TYPHI.

(a) Volg die werkwyse in 8.8. (a) behalwe dat die geïnokuleerde selenietboeljonfles 18-24 h lank by 37°C geïnkubeer moet word voordat 'n subkultuur daarvan op 'n plaat SS-agar gekweek word.

(b) Onderzoek die geïnkubeerde SS-agarplate vir vermoede kolonies *S. typhi* wat kleurloos voorkom.

(c) Stel die suiwerheid van die kultuur vas soos in 8.8 (c) beskryf.

### 8.10 SHIGELLA.

(a) Volg die werkwyse in 8.9 (a).

(b) Onderzoek die geïnkubeerde SS-agarplate vir vermoede kolonies shiggella-organismes wat ondeursigtig, deurskynend, deursigtig, kleurloos of lig pienk voorkom, en wat oor die algemeen glad is.

(c) Stel die suiwerheid van die kultuur vas soos in 8.8 (c) beskryf.

8.11 KLOSTRIDIA.—Plaas 10 ml van die 1:10-verspreiding van die monster (8.4) oor na 'n fles met differensiële versterkte klostridiumkweekbodem (8.3.3) en inkubeer 48 h lank by 37 °C. Onderzoek dit met 'n mikroskoop vir die aanwesigheid van grampositiewe stafies. Staaf die aanwesigheid van anaërobes deur dit op plate versterkte klostridiumagar (8.3.13) oor te plant, dit aërobie en anaërobie 48 h lank by 37 °C te inkubeer en dan enige kolonies wat ontwikkel weer mikroskopies te ondersoek. Beskou die groei van kolonies grampositiewe stafies op die anaërobie plate as aanduiding van die aanwesigheid van klostridia in die oorspronklike monster. Die aërobie plate moet weinig of geen groei toon nie. Indien klostridia aanwesig is, toets dit volgens aanneemlike tegnieke vir patogeniteit.

8.12 KOLIFORMIGE ORGANISMES.—Pipetteer 1 ml van die 1:10-verspreiding van die monster (8.4) in elk van die 5 petribakkies. Voeg by elke plaat 15 ml MacConkey-agar (8.3.6) wat gesmelt en tot 45 °C getemper is en meng goed (kyk 8.5). Laat die agar stol, etiketteer die plate, keer hulle om en inkubeer hulle 48 h lank by 37 °C. Onderzoek, tel en teken aan alle rooi kolonies met 'n diameter groter as 0,5 mm; verontagsaam die res. Beskou al sulke kolonies as dié van koliformige organismes.

8.13 *E. COLI* I.—Indien daar in die toets in 8.12 beskryf rooi kolonies ontstaan, kweek subkulture van elke kolonie in (a) 'n fles MacConkey-boeljon (8.3.7) en (b) 'n fles triptonwater (8.3.17) wat vooraf tot by 44 °C verhit is. Inkubeer die subkulture oornag by 44°±0,25 °C in 'n waterbad.

### 8.8 SALMONELLA.

(a) Transfer 20 g of the sample (8.4) to a previously tared sampling bottle. Add enough lactose broth (8.3.5) to give a 1:10 (m/v) dispersion. Macerate as in 8.4 and incubate at 37 °C for 6 h. Using a loop of platinum wire of 4 mm internal diameter transfer two loopfuls of this culture to 10 ml of selenite broth (8.3.14) and incubate this culture at 43 °C for 18-24 h. Streak a loopful of the culture over a plate of SS agar (8.3.15) in such a way as to assist growth of separate colonies. Incubate the plate at 37 °C for 18-24 h.

(b) Examine the plate for presumptive colonies of *Salmonella spp* which appear as colourless colonies.

(c) Establish the purity of the culture by picking off 10 suspected salmonella colonies or all such colonies (whichever is less), streaking each colony over the surface of a MacConkey agar plate (8.3.6), incubating at 37 °C for 18-24 h, and confirming the identity of the organisms by acceptable biochemical and serological techniques.

### 8.9 SALMONELLA TYPHI.

(a) Use the procedure described in 8.8 (a) except that the inoculated selenite broth bottle shall be incubated at 37 °C for 18-24 h before subculturing on to a plate of SS agar.

(b) Examine the incubated SS agar plate for presumptive colonies of *S. typhi* which appear as colourless colonies.

(c) Establish the purity of the culture as described in 8.8 (c).

### 8.10 SHIGELLA.

(a) Use the procedure described in 8.9 (a).

(b) Examine the incubated SS agar plate for presumptive colonies of shigella organisms which appear as opaque, translucent or transparent, colourless or pale pink colonies which generally are smooth.

(c) Establish the purity of the culture as described in 8.8 (c).

8.11 CLOSTRIDIA.—Transfer 10 ml of the 1:10 dispersion of the sample (8.4) to a bottle of differential reinforced clostridium medium (8.3.3) and incubate at 37 °C for 48 h. Using a microscope examine for the presence of gram-positive rods.

Confirm the presence of anaerobes by subculturing on to plates of reinforced clostridium agar (8.3.13), incubating both aerobically and anaerobically at 37 °C for 48 h, and examining microscopically any colonies which develop. Consider the growth of the colonies of gram-positive rods on the anaerobic plate as showing the presence of clostridia in the original sample. The aerobic plate should show scanty or no growth. If any clostridia are present, test them for pathogenicity by acceptable techniques.

8.12 COLIFORM ORGANISMS.—Pipette 1 ml of the 1:10 dispersion of the sample (8.4) into each of 5 petri dishes. To each plate add 15 ml of MacConkey agar (8.3.6) that has been melted and tempered to 45 °C, and mix well (see 8.5). Allow the agar to set, label the plates, invert them, and incubate them at 37 °C for 48 h. Examine, count and record all red colonies of diameter greater than 0,5 mm ignoring all others. Regard all such colonies to be those of coliform organisms.

8.13 *E. COLI* I.—If any red colonies are obtained in the test described in 8.12, subculture each colony into (a) a bottle of MacConkey Broth (8.3.7) and (b) a bottle of tryptone water (8.3.17) that have been preheated to 44 °C. Incubate the subcultures overnight at 44° ± 0,25 °C in a water bath.

Indien die MacConkey-boeljon die ontstaan van suur, soos blyk uit geel verkleuring, en gas toon en die tryptonwater 'n rooi kleur toon wanneer dit met 0,1-0,5 ml Kovacreagens (8.3.4) gemeng word deur dit versigtig te skud, neem aan dat die kolonie *E. coli* I is.

**8.14 HALOFILIESE ORGANISMES.**—Pipetteer 1 ml van die 1:10-verspreiding van die monster (8.4) in elk van twee petribakkies. Voeg by die inhoud van elke bakkie ongeveer 20 ml gemodifiseerde voedingsoutagar (8.3.8); meng deeglik en laat die agar stol. Inkubeer die bakke 14 dae lank by 37 °C. Haal die plate uit die inkubator na inkubasie en tel die getal kolonies op die plate, teken die resultate aan. Bereken die totale telling halofiliese organismes deur die gemiddelde van die afsonderlike tellings vir elke plaat met die betrokke verdunningsfaktor (10) te vermenigvuldig.

If the MacConkey broth shows the production of both acid, as indicated by yellow coloration, and gas, and the tryptone water gives a red coloration when mixed by gentle shaking with 0,1 to 0,5 ml of Kovacs reagent (8.3.4), consider the colony to be *E. coli* I.

**8.14 HALOPHILIC ORGANISMS.**—Pipette 1 ml of the 1:10 dispersion of the sample (8.4) into each of 2 petri dishes. To each of these add about 20 ml of modified nutrient brine agar (8.3.8); mix well and allow the agar to set. Incubate the dishes at 37 °C for 14 days. After incubation, remove the plates from the incubator, and count the colonies on the plates. Record these results. Calculate the total count of halophilic organisms by averaging the individual counts on each plate and multiplying this average count by the dilution factor involved (10).